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Age related development of respiratory abnormalities in non-index α-1 antitrypsin deficient studies☆

Jayne Holme [a,b], James A. Stockley [c,d], Robert A. Stockley [c,d,e,*]

[a] University Hospital of South Manchester NHS Foundation Trust, Manchester, UK
[b] North West Lung Centre, Wythenshawe Hospital, Southmoor Road, Wythenshawe, Manchester, Greater Manchester M23 9LT, UK
[c] University Hospital Birmingham NHS Foundation Trust, UK
[d] ADAPT Project Office 4, Outpatient Department Area 3, Lung Function and Sleep Department, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Edgbaston, Birmingham B15 2WB, UK
[e] University of Birmingham, UK

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Summary
Background: The role of lung function monitoring in subjects identified as having asymptomatic alpha-1 antitrypsin deficiency (AATD) is uncertain. We investigated for the first time the age these tests start to deviate from results expected in a healthy population with particular reference to the group with the best prognosis (non-smokers), and the order in which this occurs.
Methods: Spirometry, gas transfer, health status, and CT densitometry for upper and lower zones were examined in relation to age, gender, ascertainment method and smoking in 591 PiZ AATD subjects using two methods. Firstly, determining the earliest age group at which >50% of subjects consistently had actual test results worse than the healthy population mean by data observation, and secondly predicting the age when this occurred using a logistic regression model.
Results: Both methods produced similar results. For non-index subjects, gas transfer and health status deviated from normal before the age of 16, followed by upper zone densitometry and FEV_1:FVC ratio (age 29), and finally lower zone densitometry and FEV_1 (age 37). This order was similar in non-index never smokers, but occurred later (from the age of 29–63).

☆ Work performed at: University Hospital Birmingham NHS Foundation Trust, Birmingham, UK.

* Corresponding author. University Hospital Birmingham NHS Foundation Trust, UK. Tel.: +44 0121 371 2000; fax: +44 0121 371 3887.
E-mail addresses: jayne.holme@nhs.net (J. Holme), James.stockley@uhb.nhs.uk (J.A. Stockley), rob.stockley@uhb.nhs.uk (R.A. Stockley).

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Conclusions: Gas transfer, health status and CT densitometry deviate from normal from the mid-teens (up to 30 years prior to conventional spirometry) in AATD.

Introduction

Alpha-1 antitrypsin deficiency (AATD) is a genetic condition predisposing to chronic obstructive pulmonary disease (COPD).[1] Alpha-1 antitrypsin (AAT) is a glycoprotein[2] produced mainly in hepatocytes,[3] and released in the bloodstream to the lung where it is the principal inhibitor of serine proteases, such as neutrophil elastase.[4] In PiZ AATD, a point mutation causes an abnormal AAT protein to be produced, which is prone to polymerisation[5] and subsequent blockage of the terminal secretory pathway in hepatocytes.[6] This leads to retention of most of the protein in the liver and only a small percentage reaches the circulation and lung.

Neutrophil elastase plays an important role in host defence by catalysing the breakdown of micro-organisms that can lead to infection in the lung, and in the clearance of necrotic lung tissue.[7] However, if left unopposed in the lung (as in PiZ AATD), it can also cause damage similar to that seen in COPD, including alveolar destruction leading to emphysema,[8] loss of ciliated epithelium,[9] squamous cell metaplasia,[10] reduced ciliary beat frequency[11] and mucous gland hyperplasia.[12]

Furthermore, the small amount of PiZ AAT that does reach the circulation and subsequently the lung is approximately five times less potent as an inhibitor of neutrophil elastase compared with the normal PiM type of AAT protein[13–16] decreasing its protective role even further.

Despite this, the development of lung disease is not inevitable, but the risk of developing disease,[17] its' progression[18] and mortality[19] are greatly increased by smoking.

Early detection and monitoring is important to identify subjects who are developing lung disease, particularly to emphasise smoking cessation and introduce treatment, especially in light of recent findings from a randomised controlled trial of augmentation therapy, which showed some benefit.[20] At present most patients are only identified after seeing several physicians over many years by which time lung damage is well established.[21]

The World Health Organisation (WHO) recommended that all patients with adult onset asthma or COPD should be tested.[22] However, this also delays diagnosis until disease and symptomatology are established. The alternative is neonatal screening although this raises some ethical issues. Nevertheless such an approach was initiated in Sweden in 1972 and 127 PiZ subjects were identified from 200,000 infants. Subjects responded positively to advice about the hazards of smoking,[23] with little psychological impact. These subjects are undergoing regular monitoring of FEV_1 and to-date no group change has been identified in the subjects who are now in their 30s, although 42% report breathlessness.[24]

The Swedish data suggests spirometry may be insensitive to early change and we have identified subjects with established emphysema and reduced gas transfer but normal spirometry,[25] suggesting that other tests may be more informative.

The UK database of AATD subjects (ADAPT) includes smokers, non-smokers, index (those identified to have alpha-1 antitrypsin deficiency following investigation of relevant symptoms) and non-index (those identified only as a result of family or population screening) subjects who have undergone extensive assessment including spirometry, gas transfer, high resolution computed tomography (HRCT) scanning and health status. We related these parameters to age to determine when they deviated from normal values in order to provide a more informed approach to monitoring for early lung disease especially for those with the best disease prognosis (non smoking non-index cases).

Methods

The first 591 PiZ ADAPT subjects in the UK National database were included, and data from their baseline visit analysed. FEV_1, the ratio of FEV_1 to forced vital capacity (FEV_1:FVC) and carbon monoxide transfer factor corrected for alveolar volume (KCO) were measured and expressed as a percentage of predicted as previously described.[25]

An HRCT had been performed for 563 subjects, and density mask analysis was available for 368, to determine the proportion of computed tomography (CT) voxels with a density of less than −910 HU (Houndsfield Units) in the upper and lower zone, as described previously.[25] The presence or absence of visible emphysema on CT was determined using standard radiological criteria. Subjects completed a St Georges Respiratory Questionnaire (SGRQ) and demographic data, including smoking history and the reason for initial diagnosis of AATD were recorded.

The proportions of subjects with abnormal results was taken as those with an FEV_1, FEV_1:FVC and KCO of $<80\%$ predicted. There are no defined reference ranges for SGRQ scores, upper zone voxel index (UZVI) or lower zone voxel index (LZVI), so we calculated the proportion of subjects with a score greater than 1.96 standard errors above the mean data derived from a normal population,[26,27] based on a theoretical reference range encompassing values obtained in 95% of healthy subjects. (Table 3 in the Online Supplement).

The age at which test parameters start to decline was determined by 2 methods. Firstly, the cohort was split into 5-year age strata, and the proportion of subjects in each stratum who had values worse than the healthy population mean was determined for each measure. The earliest age group in which this proportion was consistently $>50\%$ was determined.

Secondly, a mathematical model was constructed including all subjects, using forward logistic regression (SPSS 12.0.1 for Windows, SPSS, Chicago, IL), with FEV_1 $<100\%$ predicted as the dichotomous dependant variable, and age, gender, smoking status and method of ascertainment as co-

variates. Using the model, an equation was produced to determine the probability of the FEV_1 being <100% predicted, expressed in terms of the covariates stated above. Coefficients for these covariates were inserted into the equation to represent the various methods of ascertainment and smoking statuses and genders. By definition, in a healthy population, it is expected that half of subjects will have an FEV_1 <100% predicted. We therefore inserted different ages into the equation to determined the earliest age at which the probability of the FEV_1 being <100% predicted was consistently greater than 0.5 (i.e. started to deviate from normal).

Similar models were constructed for FEV_1:FVC, KCO, SGRQ total score, UZVI and LZVI being less than or greater than the relevant mean for a healthy population as the dichotomous dependant variables. The mean values used for the healthy population were 100% predicted for FEV_1:FVC and KCO, and previously published normal values related to age for the SGRQ.[26] No normal values for density mask analysis at a threshold of −910 HU have been published. Therefore, previously published values determined using a GE Prospeed CT scanner with a density mask analysis threshold of −912 HU, in subjects who had never smoked and had no evidence of lung disease on pulmonary physiology and HRCT were used.[27]

Data were compared between index & non-index subjects (identified by family screening) and subjects who had smoked or not using SPSS 12.0.1 for Windows (SPSS, Chicago, IL). The $\chi 2$ and Fishers exact tests were used to compare categorical data, the one-way ANOVA test was used if continuous data was parametric and the Kruskal–Wallis test was used for non-parametric data.

Subjects gave written informed consent, and the study was approved by South Birmingham Research and Ethics Committee.

Results

Demographic data is shown in Table 1 and the proportion of subjects with abnormal physiological, radiological and health status parameters (see Methods) are shown in Table 2 for index and non-index subjects depending on the smoking status.

Age at which test parameters start to deviate from normal

5-year age strata method

The numbers of subjects in each 5 year age strata are shown in Table 4 (Online Supplement). All parameters had deviated from the mean normal value between the ages of 20 and 30 (Fig. 1). However, index cases accounted for 75% of subjects and (by definition) were likely to have had abnormal test results on presentation. The analysis of non-index cases alone was different, with the FEV_1 deviating from normal in the age range 40–45 whereas other parameters deviated in the 20–25 and 25–30 age cohorts (Fig. 2).

Logistic regression method

Gender was not a determinant of any variable so was not included in any predictive equations. The equations generated for each variable are shown in Table 5 (Online Supplement).

Most index cases had a least one physiological (99.5%), radiological (98.2%) or health status (99.3%) parameter worse than the healthy population mean at their first visit, which is reflected in the ages at which deviations from normal were predicted to occur. These predicted ages were understandably less than the age of the youngest patient at their baseline visit, because most of the data had to be derived by extrapolation. Therefore, we were unable to use this model to determine with any confidence the ages at which test parameters started to deviate from normal in the index subjects as by definition they had already presented with symptoms.

However, the results obtained for non-index cases do not involve data extrapolation but utilise primary information. The ages at which each variable is predicted to deviate from normal are shown in Fig. 2 for all non-index cases. The SGRQ and KCO are predicted to deviate from normal before the age of 16. Following this, UZVI and FEV_1:FVC were predicted to deviate from normal at age 29, then LZVI and FEV_1 (age 37).

To explore the model further in non-index subjects we repeated the logistic regression analysis with smoking

Table 1 Demographic, physiological, radiological and health status data. Data are presented as mean (±SD) if parametric and median (IQR) if non-parametric unless otherwise stated.

	Non-index never smoked (n = 54)	Non-index ex/current smokers (n = 95)	Index never smoked (n = 90)	Index ex/current smokers (n = 352)
Age	44.8 (13.8)	46.9 (11.4)	60.5 (11.4)[a,b]	49.7 (9.1)[a,c]
Male gender n (%)	18 (33.3%)	49 (51.6%)[a]	53 (58.9%)[a]	227 (64.5%)[a,b]
Pack years smoked	N/A	12.0 (5.0–21.5)	N/A	21.0 (12.8–28.0)
FEV_1% predicted	102.9 (26.7)	76.3 (29.3)[a]	65.2 (28.5)[a,b]	43.2 (19.6)[a,b,c]
FEV_1:FVC% predicted	95.0 (20.9)	71.9 (23.2)[a]	63.3 (23.7)[a,b]	45.4 (16.9)[a,b,c]
KCO% predicted	91.0 (20.9)	77.4 (21.5)[a]	73.6 (24.2)[a]	62.3 (19.8)[a,b,c]
SGRQ total score	22.4 (20.2)	37.5 (24.3)[a]	42.2 (17.7)[a]	54.2 (18.0)[a,b,c]
Upper zone voxel index (%)	16.1 (13.9) (n = 35)	20.7 (15.4) (n = 58)	28.9 (17.1)[a,b] (n = 47)	35.9 (16.4)[a,b,c] (n = 227)
Lower zone voxel index (%)	19.5 (16.6) (n = 35)	31.7 (19.8)[a] (n = 58)	40.0 (22.1)[a] (n = 47)	51.9 (16.2)[a,b,c] (n = 227)

[a] $p < 0.05$ compared with non-index never smoked.
[b] $p < 0.05$ compared with non-index ex/current smokers.
[c] $p < 0.05$ compared with index never smoked.

Table 2 Percentage of subjects with abnormal physiological, radiological and health status parameters at baseline.

	Non-index never smoked ($n = 54$)	Non-index ex/current smokers ($n = 95$)	Index never smoked ($n = 90$)	Index ex/current smokers ($n = 352$)
$FEV_1 < 80\%$ predicted	16	52[a]	72[a,b]	95[a,b,c]
FEV_1:FVC $< 80\%$ predicted	16	61[a]	73[a]	96[a,b,c]
KCO $< 80\%$ predicted	19	54[a]	60[a]	84[a,b,c]
SGRQ total score $>$ 1.96 SEM above mean	65	83[a]	97[a,b]	100[a,b,c]
UZVI $>$ 1.96 SEM above mean	69	79	92[a]	97[a,b]
LZVI $>$ 1.96 SEM above mean	9	36[a]	52[a]	73[a,b,c]
Emphysema on CT scan	20 ($n = 49$)	54[a] ($n = 86$)	64[a] ($n = 88$)	69[a,b] ($n = 340$)

[a] $p < 0.05$ compared with non-index never smoked.
[b] $p < 0.05$ compared with non-index ex/current smokers.
[c] $p < 0.05$ compared with index never smoked.

status as an additional covariate. The equations generated are shown in Table 6 (Online Supplement). Smoking status was related to all variables except UZVI.

For non-index never smokers (Fig. 3a) which represents the group with the best prognosis, the earliest test predicted to deviate from normal was the SGRQ and UZVI (age 29), then KCO (age 32), FEV_1:FVC and LZVI (age 50) and finally FEV_1 (age 63).

For non-index ex and current smokers (Fig. 3b), SGRQ and KCO were again among the earliest results predicted to deviate from normal (age < 16), followed by FEV_1:FVC (age 17), FEV_1 (age 24) then UZVI and LZVI (age 29).

Discussion

Using two methods (one observational and one predictive), we have demonstrated for the first time that KCO deviates from normal up to 30 years before spirometry in non-index subjects with AATD. This is associated with a deterioration in health status and CT densitometry especially in never-smokers, and may explain why a high proportion of the Swedish AATD cohort have normal spirometry yet complain of breathlessness.[24]

Previous studies suggest that spirometry becomes abnormal from age 50–65 in never-smokers[28,29] and from 25 years in smokers.[30] This is consistent with both our observations and predictions from logistic regression. Although no similar age predictions have been quoted for non-index subjects per se, Seersholm and Kok-Jensen[30] noted that non-index patients had higher spirometry values than index subjects, and FEV_1% predicted was <70% in only 11%, again in general agreement with this study.

Although there is no previous data available regarding the age at which gas transfer, health status and CT scans become abnormal in AATD, a high proportion of the Swedish cohort had breathlessness, wheeze and sputum production from age 16, despite spirometry being normal at 30 years of age,[24] suggesting that pathological changes and clinical symptomatology occur before spirometry becomes abnormal. This is consistent with our observations showing deviation of health status, KCO and, in never-smokers, CT densitometry, several years before spirometry.

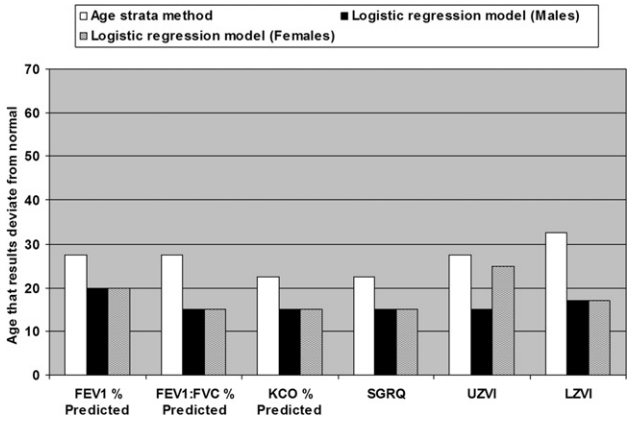

Figure 1 Age at which physiological, radiological and health status parameters had deviated from normal values for the whole study population. White columns represent values obtained using the age strata method. The black columns represent values obtained for males using the logistic regression method. The grey columns represent values obtained for females using the logistic regression method.

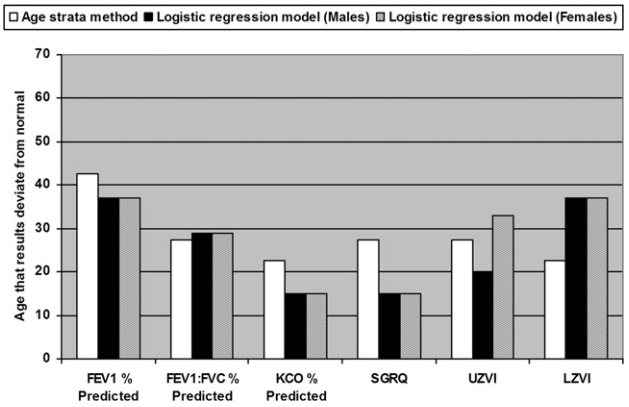

Figure 2 Age at which physiological, radiological and health status parameters had deviated from normal values for non-index subjects only. White columns represent values obtained using the age strata method. The black columns represent values obtained for males using the logistic regression method. The grey columns represent values obtained for females using the logistic regression method.

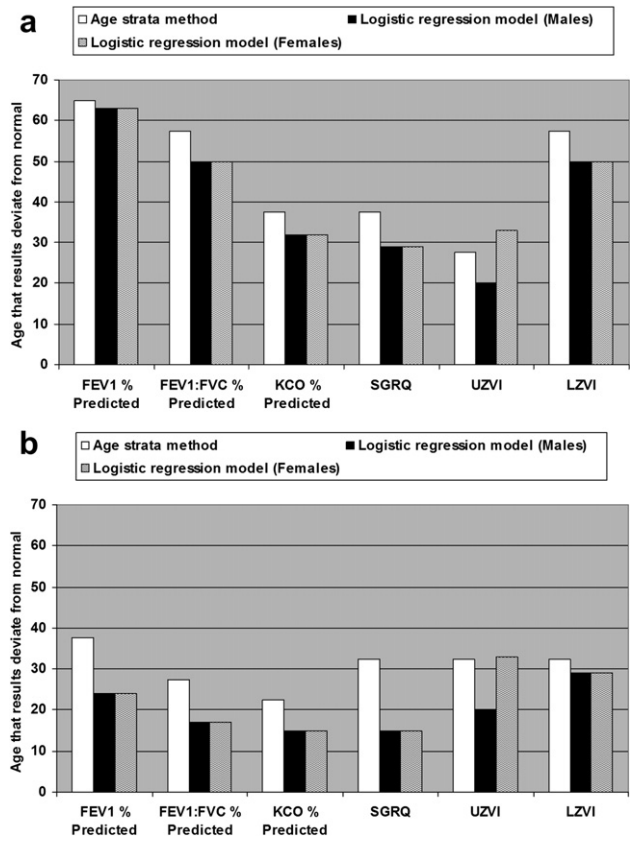

Figure 3 a: Age at which physiological, radiological and health status parameters had deviated from normal values for non-index subjects who had never smoked. White columns represent values obtained using the age strata method. The black columns represent values obtained for males using the logistic regression method. The grey columns represent values obtained for females using the logistic regression method. b: Age at which physiological, radiological and health status parameters had deviated from normal values for non-index subjects who had previously or who currently smoke. White columns represent values obtained using the age strata method. The black columns represent values obtained for males using the logistic regression method. The grey columns represent values obtained for females using the logistic regression method.

Interestingly upper zone emphysema was one of the earliest tests to deviate from normal (around the age of 29) in non-index subjects, but one of the last in smokers, in whom gas transfer and health status deviated first. It is possible that AATD in the absence of environmental factors is related initially to mild upper zone emphysema. However with the added environmental factor of smoking, lower zone emphysema develops and predominates by the time a delayed diagnosis[21] is made, leading to the observation that the classical distribution of emphysema in AATD is basal.

There are, however, some limitations to our study, most notably the design is retrospective and based on deviation from a normal range rather than a prospective documentation of trends. A cohort study such as for Swedish subjects described earlier[31] is ideal. Unfortunately the follow-up to date has been limited mainly to spirometry. Thus, it will be several decades before this study yields results which provide guidance for monitoring non-index subjects appropriately and cost effectively using FEV_1 alone. KCO, health status and CT have not been assessed in the Swedish cohort until recently and then in only a small number of subjects, thus the opportunity to obtain valuable information indicating when these tests start to deteriorate has probably been lost.

A number of our subjects did not have a CT scan. This was usually omitted in asymptomatic subjects with normal lung function or where there were anxieties about the test such as claustrophobia and radiation exposure, and thus the data may possibly represent some selection bias. CT densitometry data was also unavailable for subjects who had undergone a recent CT scan in their local hospital which was unsuitable for quantitative analysis due to acquisition methodology.

Furthermore it is difficult to make assumptions about the time course in index cases, as most had abnormal test parameters at the first visit to ADAPT. Thus any attempt to determine the age at which these became abnormal requires extrapolation beyond valid data points, rendering the results uninterpretable. However, clinically it is not relevant to determine when to test the index subjects, as by definition, they have already presented with lung disease and automatically undergo investigation.

There are few young subjects in our cohort especially index cases. Our youngest index case was 26.4 years old at diagnosis. The FEV_1 was 87.5% predicted whereas the KCO was 65% predicted, indicating that deterioration of lung function must have occurred below this age affecting KCO preferentially, again consistent with the concept of screening early using non-spirometric tests.

The non-index cases in our cohort are predominantly family members of index subjects and an influence of shared genetic susceptibility factors cannot be ruled out. However, if these are present they are likely, if anything, to result in the development of lung disease at an earlier age than non-index subjects with no affiliated index case (such as those identified by population screening), and recommendations regarding the age at which physiological or radiological screening should commence would capture the onset of disease in these patients.

We used two different methods to determine the age at which test parameters become abnormal, and obtained reasonable agreement between both. The advantages of the logistic regression model include the fact that a linear relationship between age and each test parameter is not necessarily assumed, and outlying data points have less impact on the predictions than in the 5 year age-strata method. Despite the consistency of results obtained using both methods, the true validity of this type of mathematical modelling can only be confirmed by a future prospective study. However the data does provide guidance to suggest this should include more extensive physiological testing especially in early adulthood for subjects identified as having AATD.

Both methods rely on using 'normal' values for a healthy population for each test parameter. For physiology testing, these values are robust and used frequently.[32] However, little data is available regarding 'normal' values for the SGRQ[26] and no normal data for CT densitometry using the −910 HU threshold is available. We have therefore used

data from a study reporting a similar but slightly less sensitive threshold of −912 HU although with a GE Prospeed CT scanner[27] as in our study. This is the best estimate of normal values that can be applied to the current study. Nevertheless, these slight uncertainties should be borne in mind when interpreting the results, and further study of the use of CT scans and health status scores is warranted. However, the consistency with predicted deviation from normal of KCO suggests the data for densitometry is likely to be a close estimate.

Importantly we have clearly demonstrated that KCO, health status and UZVI (in never-smokers) deviate from normal up to 30 years prior to spirometry in non-index subjects with AATD. Gas transfer deviates from normal in the teens for smokers and the early 30s for never-smokers. Based on these data, we recommend that screening for the development of lung disease should be undertaken in the 20s for non-index never smokers, and the teens for smokers. KCO is central to this assessment, and in addition to spirometry, consideration should also be given to utilising health status and CT measures, although radiation exposure may restrict the use of the latter. It seems logical that when KCO is deteriorating, subjects with AATD should undergo lifestyle modification and possibly start effective therapies such as augmentation, where available, that has been shown to influence emphysema progression(20) prior to the development of FEV_1 impairment.

This data challenges the current understanding of COPD, its' severity and current treatment guidelines,[33,34] as these are primarily based on spirometry, which becomes impaired late in the natural history of AATD. It therefore seems logical to recommend at least lung function testing and monitoring in subjects known to have AATD from the late teens and to include gas transfer as part of this routine follow up to determine whether the data is changing faster than expected for age. Similar studies of the natural history of usual COPD in smokers should be considered, as this may also identify disease at an earlier stage, facilitating the introduction of appropriate preventative therapy before airflow obstruction develops.

Conflict of interest

JH has received funding to attend international conferences from Altana Pharma and Astra Zeneca. JH has received payment for assisting in the organisation of an educational event from Astra Zeneca. JH has received lecture fees from Astra Zeneca and GSK.

JAS has no conflicts of interest to declare.

RAS has received fees for consultancy as part of an advisory board from GSK, Boehringer, Roche, Schering Plough and MSD. Lecture fees have been paid to RAS by Talecris and GSK. RAS has received industry sponsored non-commercial grants from AZ, Altana and Talecris, to cover research costs and staff salaries.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2012.12.003.

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